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Patents ADP number *(if you know it)* 07319379001

If the applicant is a corporate body, give the country/state of its incorporation

UNITED KINGDOM

4. Title of the invention

ANTIVIRAL COMPOSITION

5. Name of your agent *(if you have one)*

A A THORNTON & CO

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

235 HIGH HOLBORN
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Continuation sheets of this form	
Description	8
Claim *(s)*	1
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A. A. Thornton & Co. 12 MARCH 2004

12. Name and daytime telephone number of person to contact in the United Kingdom: Philip A. CURTIS - 020 7440 6860

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ANTIVIRAL COMPOSITION

The present invention relates to an antiviral composition.

It is known that a number of natural products have insect repellent properties. Citriadora oil obtained from various species of eucalyptus is one example of such a natural product, citronella oil which is obtained from certain grasses is another. We have previously investigated certain insect repellent natural products and have found that the insect repellent properties are in a fraction rich in p-menthane-3,8-diol (PMD). This is described in our GB-A-2282534. In GB-A-1315625, there is described the use of certain p-menthane diols, but not PMD, to provide a physiological cooling effect. EP-B-1204319 describes the use of PMD as a general antiseptic. We have now found, very surprisingly, that PMD also possesses the totally unrelated quality of antiviral properties.

According to one aspect of the invention, we provide the use of PMD in the manufacture of a medicament for use as an antiviral agent.

It is to be understood that the term "virucidal" means any compound that destroys viruses. It is also to be understood that the term "anti-viral agent" means an agent which inhibits or stops the growth and reproduction of viruses, or which destroys viruses. The use of PMD in the present invention may be virucidal.

The PMD for use in the present invention may be derived from a natural source or may be synthetic, or a mixture of the two. A preferred source of natural PMD is the lemon eucalyptus plant. Synthetic PMD may be obtained by any route, for example, such as described by Zimmerman and English in J.A.C.S. 75 (1953) pp 2367-2370.

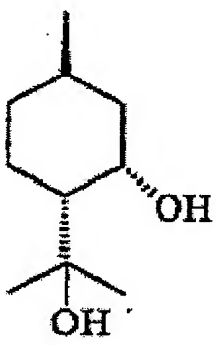
The PMD for use in the present invention may be a substantially pure form of the compound, or a crude extract, for example from a natural source. An example of a crude extract is a PMD-rich extract derived from lemon eucalyptus. The PMD can be produced by cyclisation of citronellal which is present in high concentration in lemon eucalyptus oil (approximately 75% by weight). We have obtained a PMD-rich extract from the lemon eucalyptus oil which includes both geometric isomers of PMD usually at about 64% by weight. The crude extract also includes citronellol and isopulegols plus certain other minor components.

According to a further aspect of the invention, there is provided the use of a PMD-rich extract containing composition, which extract is derived from natural lemon

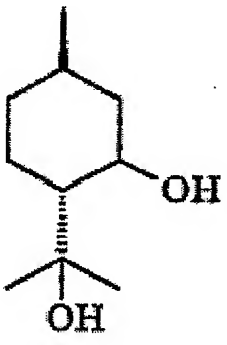
eucalyptus oil, as an antiviral agent. An example of this sort of crude extract is available under the trade mark "Citriodiol".

A composition for use in accordance with the invention can comprise PMD and a carrier. PMD is poorly soluble in water, so that it is preferred to use an oil as a carrier, or use a solvent, such as alcohol, for waterbased compositions.

It is known that PMD exists in two geometric isomeric forms, namely the cis and trans isomers, and that there are two enantiomers for each geometric isomer.



trans



cis

Our experimental work is based on the 98% pure cis isomer. It will be understood, however, that the claimed activities for PMD are common to all its isomeric forms. Thus, the PMD may be used in the form of a single pure cis or trans isomer, or a mixture of the isomers, such as a 50-50 mixture of the isomers.

In a further aspect of the invention, the composition for use in the invention comprises only one of the isomers of PMD, with a carrier therefor.

It is a further aspect of the invention that the relative amounts of cis: trans PMD isomers in the compositions for use in the present invention are varied as desired. This can be done by mixing previously separated isomers in the appropriate ratio, or by adjusting the ratio in a mixture of naturally occurring or synthetic source.

In tests we have found that PMD is effective against an influenza virus A/Sydney/5/97. In a further aspect, therefore, the invention provides the use of PMD in the manufacture of a medicament for use as an antiviral agent against an influenza virus A/Sydney/5/97.

The uses of the present invention may be adopted in sanitizing a surface, for example in a hospital room or ward. In such cases PMD is applied to the surfaces. The PMD is preferably either in solution or as an emulsion in suitable liquid carriers. Most desirably, the PMD is formulated for spray application. For example, the PMD or Citriodiol can be dissolved in a suitable solvent or solvent mixture. For example, in one embodiment, the PMD may be provided in the form of a nasal spray; in another embodiment, the PMD may be provided in the form of a spray for telephones.

In a one mode of application, the spray is an electrostatic spray. For electrostatic spraying, the solvent or solvent system will need to be appropriate for electrostatic spraying, as will be clear to those skilled in the art. I prefer to use a mixture of conductive and nonconductive solvents to achieve a sprayable solution with the appropriate electrical resistivity for the spray nozzle in question, but suitable single solvents can of course be used. Charged particles of the composition including PMD are projected as a fine mist and because all the particles carry a similar, for example positive, charge they repel each other, but are attracted to an oppositely charged surface. By this means of spraying, a very good coverage of the composition on the surface may be obtained. Devices for electrostatically spraying the composition for use in the invention will be known to the person skilled in the art.

A spray may also be used, for example, for dispensing a composition including PMD onto a hand (or other part) of a person. The actuation of the dispenser may be by means of an infra-red sensor, for example, so that the person need not contact a surface, and thereby risk the transfer of microbes to or from their hand. Electrostatic spray application to a hand may be used, with advantage, where a substantially uniform coverage of antiseptic is particularly important e. g. to a surgeon during "scrubbing up" before surgery.

To increase the likelihood of the charged particles covering the skin surface, desirably the electrostatic spray nozzles may be arranged to spray into the interior of a cabinet or container as the hand is introduced therein.

The liquids for applying to a surface, by spraying or otherwise, in accordance with the invention may contain, apart from the solvent (s) and/or other liquid carrier (s), other components as necessary or desirable for the intended purpose. Thus, second or further antiviral agents may be included, as may surfactants, fragrances etc. In general, the compositions may be identical to known compositions for the purpose except that

they contain PMD in addition to, or in whole or part substitution for one or more of, the other ingredients.

The amount of PMD can vary widely. Although our tests have showed that PMD levels of 0.5% w/v and less impart no virucidal effect against influenza virus A/Sydney/5/97, it may have an effect against other viruses at lower concentrations. PMD may also be included as an anti-viral agent in household detergents, cleansers and creams, for example, washing powders or conditioners and hand gels.

Again, the PMD may be included in what are otherwise standard or known compositions for the purpose concerned. The PMD may be an extra ingredient or in partial or complete replacement of a standard ingredient. The compositions may already contain an antiviral agent and the PMD is added to give an extra anti-viral effect.

Furthermore, PMD may be impregnated into household objects which may be prone to microbial infestation and so risk infecting inhabitants, e. g. dishcloths, plastic soap dishes, surfaces used for the preparation of food.

For these purposes, the PMD may be included during manufacture of the object, e. g. in mixtures for plastics mouldings or the like, or it may be applied to the object after manufacture, e. g. by soaking dishcloths in PMD. The presence of the PMD at the surface of the object will provide the desired antiseptic effect. This is particularly useful for work surfaces, although of course such surfaces-can also be regularly treated with PMD as by spraying or otherwise.

A composition including PMD can also be used in medicine. For example, it can be applied to broken skin, or to internal mucous membranes. It may be an ingredient in throat lozenges or pastilles or other products for ingestion. In this aspect, the invention provides PMD for use as an antiseptic, antibiotic, bactericide or fungicide. In medical uses the PMD may be formulated with the carrier as a cream, or, as mentioned above, as a throat lozenge or pastille. A composition including PMD may be applied to the accessible inner surfaces of the nose in order to control or eliminate viruses which may cause regular systemic effects. Another specific medical use is in wound irrigation during surgery, e. g. surgery conducted on the peritoneal cavity.

As will be evident to those skilled in the art, there are a very large number of medical uses of PMD as an antiviral or virucidal agent. In general, new formulations for these purposes are not required: it is adequate and satisfactory to take a known or

standard composition and include the PMD therein. Alternatively, one or more ingredients may be replaced by the PMD as appropriate. Those skilled in the art will well know the make-up of the various compositions and no further particular description thereof is given here.

PMD is the active ingredient in the insect repellent sold under the trade name "Mosiguard"™. Tests have already been conducted show regulatory authorities that PMD is not toxic, and the Mosiguard insect repellent has been marketed for several years and there has been no report of any significant toxicity thereof. Potentially, therefore, the medical uses of PMD may be topical or systemic. Systemic administration may be by way of an oral dosage form or by a parenteral route, such as by intra-venous injection.

In general, PMD is used in accordance with the invention in a wide variety of vehicles, depending on the particular use intended. The vehicles may, for example, include solids, liquids, emulsions, foams and gels.

Typical vehicles include aqueous or alcoholic solutions, oils, fats, fatty acid esters, long chain alcohols and silicone oils, finely divided solids such as starch or talc, cellulosic materials and aerosol propellants. Topical compositions include perfumes, powders and other toiletries, lotions, liniments, oils and ointments, for example. Toiletries generally include after shave lotions, shaving soaps, lipstick, creams, foams, toilet water, deodorants, antiperspirants, solid colognes, toilet soaps, bath oils and salts, shampoos, face and hand creams, cleansing tissues, mouthwashes, eye drops, for example. Medicaments and allied compositions include, for example, ointments, lotions, decongestants and throat lozenges. The amount of PMD present in the compositions will be selected to give the desired effect but we believe that generally up 5.0 wt%, preferably from 0.1 to 5.0 wt % will be satisfactory. Greater amounts can be used. A particularly preferred concentration is from 1.0 to 3.0wt%. especially about 2 wt%.

A PMD-rich extract may be obtained from PMD-containing material, such as the leaves of a eucalyptus plant. A preferred source of PMD rich extract is obtained by stirring eucalyptus citriadora oil derived from the plant with dilute sulphuric acid (usually 5% sulphuric acid), as previously explained in our GB-A-2282534.

In order that the invention may be fully understood, the following Procedures are given by way of illustration only.

Procedure 1

Procedure for the acute toxicity assay of PMD

The toxicity of PMD at the following concentrations in cell maintenance media was determined on a cell line with MDCK cells:

- 2.5mg/ml (0.25% w/v)
- 5mg/ml (0.5% w/v)
- 20mg/ml (2% w/v)

A cell only control was also implemented by following the same procedure (steps 2-6), but substituting PMD with cell maintenance media.

Toxicity was determined by toxicity-induced CPE (cytopathic effect) observations, which was visually scored using microscopy techniques. Toxicity-induced CPE is characterised by burst or rounded cells, which have become dissociated from their neighbouring cells, or the presence of cellular debris. Toxicity-induced CPE was scored as positive (toxicity observed) or negative (no toxicity observed).

(1) 200µl of each PMD dilution was added to 200µl of cells (at 2 x 106 cells/ml) and the reaction incubated for 5 minutes at room temperature.

(2) The reaction was terminated by adding 3.6mls of cell maintenance media appropriate to the cell line.

Note: termination of the reaction is due to the addition of cell maintenance media, which dilutes the reaction 10-fold.

(3) 100µl of the terminated reaction was added to the relevant wells on 48-well plates and incubated for 24 hours at 37°C, 5% CO2.

Note: the remaining terminated reaction was measured for levels of pH.

(4) The cells in the 48-well plate were typsinised and a viable cell count was performed using the Trypan blue dye. The percentage of viable cells was used to determine the toxic concentration of PMD in comparison to the cells only contr

Procedure 2

Procedure for the virucidal assay of PMD

The virucidal assay was carried out using four different concentrations of PMD, which do not exhibit toxicity (based on the data obtained from the procedure described in 1).

The four concentrations are summarised in the following table.

Concentration (w/v)	0.1	0.25	0.5	2
Concentration (mg/ml)	1	2.5	5	20

The stock virus was used at a titre greater than 10^4 $TCID_{50}$/ml.

The appropriate positive anti-viral control compound was citric acid.

The presence and absence of viral infection was determined by infection-induced CPE observation, which was visually scored using microscopy techniques. Infection-induced CPE differs between viruses, but is normally characterised by ballooning or rounded cells that remain attached to their neighbouring cells. It was scored as positive (infection apparent) or negative (infection not apparent).

(1) MDCK cell lines were cultivated according to the current Retroscreen Virology Ltd. SOP onto 96-well plates.

(2) 40µl of A/Sydney/5/97 virus was added to 360µl of each PMD dilution and citric acid.

Note: The virus was diluted 10-fold in this step.

(3) The reactions were incubated at room temperature for the following contact times:

a. 10 seconds
b. 30 seconds
c. 1 minutes
d. 5 minutes

(4) At each contact time point, the reaction was terminated by adding 3.6ml of infection media appropriate to the cell line.

Note: termination of the reaction is due to the addition of infection media, which dilutes the reaction 10-fold.

(5) 100µl of the terminated reaction was added to the first column of 96-well plates (prepared in point 1) and titrated across the plate following a 1/10 dilution series.

Note: the remaining terminated reaction was measured for levels of pH.

(6) The cells were incubated for 3-5 days at 37°C, 5% CO_2.

(7) CPE was scored daily on the plates to determine the presence or absence of infection. The reduction in viral titre (as a result of anti-viral activity of PMD and the positive control compound, citric acid) was determined.

The results showed that using an influenza virus A/Sydney/5/97 on MDCK cells, there was no viral replication as evidenced by cell survival with 2% w/v PMD in the culture medium. At lower concentration (0.5%, 0.25% and 0.1%) cells were killed indicating no virucidal effect at these levels.

CLAIMS

1. The use of p-menthane-3,8-diol (PMD) for the manufacture of a medicament for use as an antiviral agent.

2. The use of PMD as an anti-viral agent in non-therapeutic, non-surgical and non-diagnostic applications.

3. The use according to claim 1, wherein the PMD is used to treat influenza virus A/Sydney/5/97.

4. The use of PMD to denature influenza virus A/Sydney/5/97.

5. The use of PMD to denature viruses.

6. The use according to any preceding claim, wherein the PMD is a crude or purified natural product or is a synthetic product.

7. The use according to any preceding claim, wherein the PMD is provided in the form of PMD-rich extract derived from lemon eucalyptus.

8. The use according to any preceding claim, wherein the PMD is provided in the form of a spray.

9. The use according to any preceding claim, wherein the PMD is provided in the form of a composition comprising PMD and a carrier.

10. The use according to any preceding claim, wherein the amount of PMD in the composition is at least 0.5% w/v.

11. The use of PMD substantially as herein described with reference to and as shown in the examples.

ABSTRACT

ANTIVIRAL COMPOSITION

The use of p-menthane-3,8-diol (PMD) as an antiviral agent.